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Antimicrobial Susceptibility of Clinically Relevant Gram-Positive Anaerobic Cocci Collected over a Three-Year Period in the Netherlands[▽]†

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The susceptibility of 14 species of 115 Gram-positive anaerobic cocci (GPAC) was determined for 14 antibiotics. To assure correct identification, strains were genotypically identified by fluorescence *in situ* hybridization and sequencing. Susceptibility differences (MIC_{50} and MIC_{90}) for penicillin G, clindamycin, tigecycline, levofloxacin, amoxicillin-clavulanic acid, cefoxitin, ertapenem, meropenem, metronidazole, and doxycycline were found for the three clinically most relevant GPAC species: *Finegoldia magna*, *Parvimonas micra*, and *Peptoniphilus harei*.

Gram-positive anaerobic cocci (GPAC) are part of the commensal microbiota and account for about one-third of the anaerobic isolates recovered from clinical materials (14). It is a heterogeneous group, which in the last decade has undergone an extensive taxonomic change. The species *Peptostreptococcus micros* and *Peptostreptococcus magnus* were transferred to two new genera, *Micromonas* and *Finegoldia*, respectively, with each being the only species present in their respective genus (15). The genus *Micromonas* has recently been replaced by *Parvimonas*, with *Parvimonas micra* (*Pa. micra*) being the only species present (20). Ezaki et al. (7) divided the remaining peptostreptococci into three phylogenetic groups, *Peptoniphilus* gen. nov., *Anaerococcus* gen. nov., and *Gallicola* gen. nov., with *Gallicola barnesae* being the only species present in the latter genus. The species left in the genus *Peptostreptococcus* include *Peptostreptococcus anaerobius* (*Pe. anaerobius*) and a recently described new species, *Pe. stomatis* (6). Song et al. (19) described three new species: *Peptoniphilus gorbachii* (*Pt. gorbachii*) sp. nov., *Pt. olsenii* sp. nov., and *Anaerococcus murdochii* sp. nov. The most commonly found GPAC in clinical material are *Finegoldia magna*, *Pa. micra*, *Pt. harei* (21), and *Pe. anaerobius* (22). The data on the antimicrobial susceptibility of the different species of GPAC is often based on GPAC in general, even though several authors describe a difference in antimicrobial susceptibility between species (3–5, 11, 12, 18). In these studies, the strains were identified phenotypically. However, for some species it is difficult to obtain a reliable phenotypic identification, e.g., in the past *Pt. harei* has often been misidentified as *Pt. asaccharolyticus* (21), probably due to the fact that these two species share the same biochemical characteristics (10).

In the present study, we assessed the susceptibility of 115 isolates of GPAC against 14 different antibiotics. Isolates were genotypically identified by using fluorescence *in situ* hybridization (FISH) (21) or sequencing, thus allowing more accurate insight into the distribution of susceptible and resistant strains within the different species.

MATERIALS AND METHODS

Isolates. Strains were obtained from the diagnostic laboratory of the University Medical Center Groningen and collected in the years 2002 to 2004. All strains were isolated from human clinical samples from a variety of anatomical sites, e.g., from abdominal, head and neck, and soft tissue infections. Strains were stored at −80°C and subcultured on brucella blood agar (BBA) prior to susceptibility testing.

Identification. Strains were genotypically identified by using 16S rRNA-based probes (21) and sequencing. Shortly thereafter, bacterial cells were harvested from BBA using a sterile loop and fixed in 1:1 phosphate-buffered saline (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per liter) and ethanol 96% (vol/vol). Fixed cells were spotted on slides and, if necessary, permeabilized using proteinase K. Strains were hybridized by using probes directed against *F. magna*, *Pa. micra*, *Pt. harei*, *Pe. anaerobius*, *A. vaginalis*, *Pt. asaccharolyticus*, *A. lactolyticus*, and *Pt. ivorii*. The addition of new species to the genera *Peptoniphilus* and *Anaerococcus* (19) showed that the probes directed against *A. lactolyticus* and *Pt. harei* were also positive, with *A. murdochii* and *Pt. gorbachii*, respectively (data not shown). Strains that were negative with the probes or positive with the probes directed against *A. lactolyticus* and *Pt. harei* were sequenced. DNA was isolated as described previously (2), and the 16S genes were amplified and sequenced using universal 16S rRNA-specific primers (9). Sequences were compared to those in the GenBank database by performing a BLAST search (National Center of Biotechnology Information) (1).

Susceptibility testing. The antimicrobial susceptibility using penicillin G, amoxicillin-clavulanic acid, cefotetan, cefoxitin, ertapenem, meropenem, levofloxacin, moxifloxacin, clindamycin, metronidazole, linezolid, chloramphenicol, doxycycline, and tigecycline was determined by using Etest (AB Biodisk, Sweden). Suspensions of approximately 2 McFarland standards were made in prereduced brucella broth and applied onto a prereduced BBA. All culture handlings were performed in an anaerobic chamber. Plates with Etest strips were incubated for 48 h at 37°C in an anaerobic chamber before reading the MIC. In each batch a quality control strain *Bacteroides fragilis* ATCC 25285 was included.

A difference in susceptibility was defined as at least a two-dilution-step (with one dilution step being a difference of 2-fold dilutions with a precision of a 0.5 dilution) difference between the MIC's of the different species.

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TABLE 1. MIC values determined from the quality control tests on *B. fragilis* ATCC 25285

Antibiotic	MIC (mg/liter)	
	MICs (no. of tests)	Expected MIC range[a]
Penicillin G	12 (2), 16 (7), 24 (1)	8–32
Amoxicillin-clavulanic acid	0.19 (2), 0.25 (5), 0.38 (3)	0.125–0.5†
Cefotetan	6 (7), 8 (3)	4–16
Cefoxitin	4 (1), 6 (7), 8 (2)	4–16
Ertapenem	0.125 (4), 0.19 (6)	0.064–0.25
Meropenem	0.094 (2), 0.125 (4), 0.19 (4)	0.064–0.25
Levofloxacin	1 (1), 1.5 (9)	1*
Moxifloxacin	0.19 (1), 0.25 (1), 0.38 (6), 0.5 (2)	0.125–0.5
Clindamycin	1.5 (2), 2 (4), 3 (4)	0.5–2
Metronidazole	0.25 (4), 0.38 (4), 0.5 (2)	0.25–1
Linezolid	4 (1), 6 (5), 8 (3), 12 (1)	2–8*
Chloramphenicol	6 (3), 8 (7)	2–8
Doxycycline	0.25 (3), 0.38 (5), 0.5 (2)	0.25–0.5*
Tigecycline	0.25 (2), 0.5 (2), 0.75 (6)	0.125–1*

[a] The expected range is derived from CLSI standards for *B. fragilis* for reference agar dilution testing, except as indicated: *, expected range derived from literature; and †, expected range derived from the manufacturer.

RESULTS

The quality control strain *B. fragilis* ATCC 25285 was tested 10 times with all 14 antibiotics. The obtained MICs are summarized in Table 1.

All results of the clinical isolates are summarized in Table 2 and Table 3. The MIC_{50} and MIC_{90} values were only calculated for species for which more than 10 strains were present in the study, i.e., *F. magna*, *Pa. micra*, and *Pt. harei*. Upon comparing the MIC_{50} and MIC_{90} values for these three species, *F. magna* had the highest MIC_{50} and MIC_{90} values for penicillin G, amoxicillin-clavulanic acid, clindamycin, and tigecycline. It has the highest MIC_{50} values for cefotetan, cefoxitin, meropenem, linezolid, and chloramphenicol and the highest MIC_{90} values for levofloxacin and moxifloxacin. *Pa. micra* has the lowest MIC_{50} and MIC_{90} for levofloxacin, metronidazole, and doxycycline and the lowest MIC_{90} for amoxicillin-clavulanic acid. *Pt. harei* has the highest MIC_{50} for levofloxacin and doxycycline. It has the lowest MIC_{50} and MIC_{90} for cefoxitin, ertapenem, and meropenem and the lowest MIC_{90} for chloramphenicol.

DISCUSSION

Since GPAC can show poor growth, we used a McFarland 2 inoculum. The MICs obtained with the quality control strain *B. fragilis* ATCC 25285 show that most of these values are within the expected range. Comparison between a McFarland standard 1 and 2 inoculum using the quality control strain gave the same MIC value (data not shown). However, 4 of the 10 MIC values obtained for clindamycin were just above the expected range obtained using McFarland standard 2. Since GPAC show poor growth compared to *B. fragilis*, this is not expected to affect our set of data. A practical approach is to use a higher McFarland turbidity as recommended by the manufacturer of Etest.

In the present study strains were identified genotypically, since phenotypic identification is not always reliable for all species (21). It is difficult to compare our results to other published resistance data, since authors may use different breakpoints. For example, some did use breakpoints advised by the Clinical and Laboratory Standards Institute (CLSI), while others used those advised by EUCAST. Therefore, we have chosen to base a difference in susceptibility on the MIC_{50} and MIC_{90} values, instead of the percentage resistant strains. However, the interpretation of our results using CSLI and EUCAST breakpoints is provided in the supplemental material.

The clinically most important GPAC in our study are *F. magna*, *Pa. micra*, and *Pt. harei*. The latter can be especially difficult to identify phenotypically, since its biochemical features resemble those of *Pt. asaccharolyticus* (10). In the past, *Pt. harei* was probably often misidentified as *Pt. asaccharolyticus*, resulting in limited susceptibility data for this species. Brazier et al. (4) included 44 clinical isolates of *Pt. harei* in a European study; all of them were phenotypically identified. No resistance was reported. In a susceptibility study in England and Wales (5), four clinical isolates of *Pt. harei* were included; all of these were also phenotypically identified. Resistance ($MIC > 256$) was reported to clindamycin. In our study, the MIC_{50} and MIC_{90} values for clindamycin were 0.25 and 1.5, respectively. The latter was the highest MIC found for *Pt. harei*.

Our study is the first to include *Pt. gorbachii* and *A. murdochii*, although the numbers are low. It is worth mentioning that one strain of *A. murdochii* had high MIC values for 4 of the 14 antibiotics: doxycycline, ertapenem, levofloxacin, and penicillin G.

Differences in susceptibility to antibiotics were described for *Pe. anaerobius* and *Pe. stomatis* (12). *Pe. anaerobius* has higher MIC values for amoxicillin, amoxicillin-clavulanic acid, cefoxitin, ertapenem, azithromycin, clindamycin, metronidazole, and moxifloxacin than *Pe. stomatis*; only the MIC_{90} of azithromycin and moxifloxacin was not two dilution steps higher. Brazier et al. (5) also suggest that some GPAC species are more resistant to antibiotics than others. For example, *Pe. anaerobius* had a higher MIC_{50} for tetracycline but had lower MIC values for erythromycin than did *F. magna*. Roberts et al. (18) showed that *Pe. anaerobius* has higher MIC_{50} and MIC_{90} values for amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, cefotetan, and meropenem than did *F. magna*, *Pa. micra*, and *Pt. asaccharolyticus*. Koeth et al. (11) showed that *F. magna* has a higher MIC_{50} for clindamycin as *Pa. micra* and *Pe. anaerobius*, while *Pe. anaerobius* has the highest MIC_{90} for amoxicillin-clavulanic acid.

Metronidazole is often the drug used for empirical treatment of anaerobic infections. However, GPAC strains are described which are resistant to this drug (11, 13, 16). We encountered one strain of *Pa. micra* that was resistant to metronidazole ($MIC > 256$). Microbiologists should be aware of this possibility. It is remarkable to notice the difference in susceptibility to the different antibiotics between the three most clinically important GPAC: *F. magna*, *Pa. micra*, and *Pt. harei*. Therefore, it is important to identify clinical isolates of GPAC. *F. magna* and *Pa. micra* can be reliably phenotypically identified by using a commercially available enzymatic kit such as Rapid ID 32A (21). However, *Pt. harei* cannot be

TABLE 2. MICs and range for GPAC against 14 antibiotics

Organism (no. of strains)[a]	Antibiotic	MIC (mg/liter)		
		Range	MIC_{50}	MIC_{90}
F. magna (31)	Penicillin G	0.023–0.38	0.125	0.25
	Amoxicillin-clavulanic acid	0.094–2	0.25	0.5
	Cefotetan	0.25–4	2	2
	Cefoxitin	0.38–3	1	1.5
	Ertapenem	0.016–0.19	0.064	0.125
	Meropenem	0.064–0.25	0.125	0.19
	Levofloxacin	0.094–64	0.75	64
	Moxifloxacin	0.047–64	0.19	6
	Clindamycin	0.125–>256	1	3
	Metronidazole	0.094–1.5	0.38	1
	Linezolid	2–6	3	3
	Chloramphenicol	4–16	6	8
	Doxycycline	0.75–24	2	24
	Tigecycline	0.064–1	0.25	0.75
Pa. micra (27)	Penicillin G	0.016–0.125	0.016	0.047
	Amoxicillin-clavulanic acid	0.016–0.75	0.032	0.094
	Cefotetan	0.125–2	0.38	1.5
	Cefoxitin	0.125–3	0.5	2
	Ertapenem	0.008–0.19	0.047	0.125
	Meropenem	0.008–0.38	0.047	0.19
	Levofloxacin	0.125–3	0.25	0.5
	Moxifloxacin	0.094–1.5	0.19	0.38
	Clindamycin	0.047–2	0.38	1.5
	Metronidazole	0.032–>256	0.094	0.25
	Linezolid	0.125–3	1	3
	Chloramphenicol	0.75–6	3	6
	Doxycycline	0.047–4	0.125	1
	Tigecycline	0.016–0.38	0.064	0.125
Pt. harei (16)	Penicillin G	0.016–0.19	0.023	0.032
	Amoxicillin-clavulanic acid	0.016–0.38	0.023	0.25
	Cefotetan	0.38–8	0.5	1
	Cefoxitin	0.023–1.5	0.094	0.5
	Ertapenem	0.006–0.023	0.012	0.016
	Meropenem	0.004–0.032	0.008	0.032
	Levofloxacin	2–64	4	6
	Moxifloxacin	0.125–1.5	0.19	0.38
	Clindamycin	0.094–1.5	0.25	1.5
	Metronidazole	0.032–2	0.38	1.5
	Linezolid	0.5–2	0.75	1.5
	Chloramphenicol	1.5–4	3	3
	Doxycycline	0.064–24	8	16
	Tigecycline	0.023–0.25	0.094	0.25
A. vaginalis (8)	Penicillin G	0.016–0.094		
	Amoxicillin-clavulanic acid	0.016–0.125		
	Cefotetan	0.094–0.5		
	Cefoxitin	0.032–0.125		
	Ertapenem	0.023–0.19		
	Meropenem	0.006–0.125		
	Levofloxacin	24–64		
	Moxifloxacin	0.5–2		
	Clindamycin	0.023–>256		
	Metronidazole	0.047–0.5		
	Linezolid	0.38–1.5		
	Chloramphenicol	1.5–3		
	Doxycycline	0.125–16		
	Tigecycline	0.047–1.5		
Pt. ivorii (5)	Penicillin G	0.016–0.047		
	Amoxicillin-clavulanic acid	0.016–0.032		
	Cefotetan	0.125–1		
	Cefoxitin	0.125–0.75		
	Ertapenem	0.004–0.032		
	Meropenem	0.002–0.016		
	Levofloxacin	0.38–64		
	Moxifloxacin	0.094–64		
	Clindamycin	0.094–2		
	Metronidazole	0.094–0.25		
	Linezolid	0.19–2		
	Chloramphenicol	1–3		
	Doxycycline	0.064–16		
	Tigecycline	0.032–0.25		
Pe. anaerobius (4)	Penicillin G	0.064–2		
	Amoxicillin-clavulanic acid	0.125–4		
	Cefotetan	0.5–24		
	Cefoxitin	0.19–3		
	Ertapenem	0.032–0.75		
	Meropenem	0.023–1		
	Levofloxacin	0.38–1.5		
	Moxifloxacin	0.19–0.25		
	Clindamycin	0.032–1		
	Metronidazole	0.032–0.25		
	Linezolid	0.38–1.5		
	Chloramphenicol	1–3		
	Doxycycline	0.5–4		
	Tigecycline	0.064–0.125		
Pt. lacrimalis (4)	Penicillin G	0.016–0.125		
	Amoxicillin-clavulanic acid	0.016–0.25		
	Cefotetan	0.016–0.38		
	Cefoxitin	0.016–0.25		
	Ertapenem	0.002–0.012		
	Meropenem	0.002–0.016		
	Levofloxacin	3–8		
	Moxifloxacin	0.002–0.38		
	Clindamycin	0.016–0.38		
	Metronidazole	0.023–0.38		
	Linezolid	0.19–2		
	Chloramphenicol	0.75–3		
	Doxycycline	0.125–4		
	Tigecycline	0.023–0.25		
Pt. gorbachii (4)	Penicillin G	0.016–0.19		
	Amoxicillin-clavulanic acid	0.016–0.064		
	Cefotetan	0.5–1.5		
	Cefoxitin	0.064–0.5		
	Ertapenem	0.012–0.023		
	Meropenem	0.004–0.064		
	Levofloxacin	3–64		
	Moxifloxacin	0.19–0.5		
	Clindamycin	0.125–0.75		
	Metronidazole	0.023–0.5		
	Linezolid	0.75–1.5		
	Chloramphenicol	2–3		
	Doxycycline	0.064–0.38		
	Tigecycline	0.016–0.094		
A. murdochii (3)	Penicillin G	0.016–0.75		
	Amoxicillin-clavulanic acid	0.032–0.25		
	Cefotetan	0.75–8		
	Cefoxitin	0.125–1		
	Ertapenem	0.19–2		
	Meropenem	0.125–0.75		
	Levofloxacin	1.5–4		
	Moxifloxacin	0.25		
	Clindamycin	0.016–0.5		
	Metronidazole	0.19–0.5		
	Linezolid	0.38–0.75		
	Chloramphenicol	1–3		
	Doxycycline	0.25–16		
	Tigecycline	0.047		
At. parvulum (4)	Penicillin G	0.094–0.25		
	Amoxicillin-clavulanic acid	0.064–0.25		
	Cefotetan	2–8		
	Cefoxitin	1.5–3		
	Ertapenem	0.032–0.19		
	Meropenem	0.125–0.25		
	Levofloxacin	0.38–0.5		
	Moxifloxacin	0.19–0.38		
	Clindamycin	1.5–6		
	Metronidazole	0.19–0.5		
	Linezolid	0.75–2		

Continued on following page

TABLE 2—*Continued*

Organism (no. of strains)[a]	Antibiotic	MIC (mg/liter)		
		Range	MIC_{50}	MIC_{90}
	Chloramphenicol	4–16		
	Doxycycline	1–2		
	Tigecycline	0.064–0.5		
A. tetradius (2)	Penicillin G	0.023–0.032		
	Amoxicillin-clavulanic acid	0.032–0.064		
	Cefotetan	0.25–0.5		
	Cefoxitin	0.19–0.38		
	Ertapenem	0.094–0.125		
	Meropenem	0.094–0.125		
	Levofloxacin	2–3		
	Moxifloxacin	0.19–0.38		
	Clindamycin	1–4		
	Metronidazole	0.25–0.75		
	Linezolid	1–1.5		
	Chloramphenicol	3–3		
	Doxycycline	2–8		
	Tigecycline	0.125–0.19		
Pt. octavius (1)	Penicillin G	0.125		
	Amoxicillin-clavulanic acid	0.064		
	Cefotetan	0.5		
	Cefoxitin	0.25		
	Ertapenem	0.094		
	Meropenem	0.094		
	Levofloxacin	4		
	Moxifloxacin	0.5		
	Clindamycin	0.047		
	Metronidazole	0.38		
	Linezolid	0.75		
	Chloramphenicol	2		
	Doxycycline	0.19		
	Tigecycline	0.064		
R. gnavus (1)	Penicillin G	1		
	Amoxicillin-clavulanic acid	0.19		
	Cefotetan	32		
	Cefoxitin	4		
	Ertapenem	0.38		
	Meropenem	0.125		
	Levofloxacin	64		
	Moxifloxacin	6		
	Clindamycin	0.38		
	Metronidazole	0.094		
	Linezolid	2		
	Chloramphenicol	3		
	Doxycycline	0.25		
	Tigecycline	0.094		
A. lactolyticus (1)	Penicillin G	0.125		
	Amoxicillin-clavulanic acid	0.125		
	Cefotetan	2		
	Cefoxitin	0.5		
	Ertapenem	1		
	Meropenem	0.38		
	Levofloxacin	6		
	Moxifloxacin	0.19		
	Clindamycin	0.047		
	Metronidazole	0.25		
	Linezolid	0.38		
	Chloramphenicol	1		
	Doxycycline	0.38		
	Tigecycline	0.094		
GPAC (4)	Penicillin G	0.023–0.125		
	Amoxicillin-clavulanic acid	0.016–0.094		
	Cefotetan	1–4		
	Cefoxitin	0.125–1		
	Ertapenem	0.006–2		
	Meropenem	0.008–0.75		
	Levofloxacin	0.5–2		
	Moxifloxacin	0.064–0.38		
	Clindamycin	0.094–0.125		
	Metronidazole	0.064–0.38		
	Linezolid	0.5–1		
	Chloramphenicol	1.5–3		
	Doxycycline	0.094–1		
	Tigecycline	0.023–0.19		

[a] Genus abbreviations: *Pe*., *Peptostreptococcus*; *Pa*., *Parvimonas*; *Pt*., *Peptoniphilus*; *A.*, *Anaerococcus*; *R.*, *Ruminococcus; At., Atopobium*.

phenotypically distinguished from *Pt. asaccharolyticus* (10, 21). The combination of diminished antimicrobial susceptibility, its prevalence, and the described virulence factors (8) gives *F. magna* a special position among the GPAC.

TABLE 3. Overall resistance of GPAC against 15 antibiotics[a]

Antibiotic	MIC (mg/liter)		
	Range	MIC_{50}	MIC_{90}
Penicillin G	0.016–2	0.047	0.19
Amoxicillin-clavulanic acid	0.016–4	0.094	0.38
Cefotetan	0.016–32	0.75	3
Cefoxitin	0.016–4	0.5	2
Ertapenem	0.002–2	0.064	0.19
Meropenem	0.002–1	0.064	0.25
Levofloxacin	0.094–64	0.75	64
Moxifloxacin	0.002–64	0.25	1.5
Clindamycin	0.016–>256	0.38	2
Metronidazole	0.023–>256	0.19	0.75
Linezolid	0.125–6	1.5	3
Chloramphenicol	0.75–16	3	8
Doxycycline	0.047–24	1	16
Tigecycline	0.016–1.5	0.094	0.38

[a] The overall resistance of GPAC (n = 115) against various antibiotics is indicated.

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